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**Title:** Appetite sensations and substrate metabolism at rest, during exercise and recovery: impact of a high-calcium meal.

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**Abstract:**

The aim of this study was to investigate the effects of the calcium content of a high-carbohydrate, pre-exercise meal on substrate metabolism and appetite sensations before, during and after exercise. Nine active males participated in 2 trials in a double-blind, randomized, crossover design. After consuming a high carbohydrate (1.5 g/kg body mass) breakfast with a calcium content of either 3 (CON) or 9 mg/kg body mass (CAL), participants ran at 60%  $\text{VO}_{2\text{peak}}$  for 60 min. Following exercise, a recovery drink was consumed and responses were investigated for a further 90 min. Blood and expired gas were sampled throughout to determine circulating substrate and hormone concentrations and rates of substrate oxidation. Visual analogue scales were also administered to determine subjective appetite sensations. Neither whole-body lipid oxidation, nor free fatty acid availability differed between trials. The area under the curve for the first hour following breakfast consumption was 16% (95% CI: 0, 35%) greater for fullness and 10% (95% CI: 2, 19%) greater for insulin in the CAL trial but these differences were transient and not apparent later in the trial. This study demonstrates that increasing the calcium content of a high carbohydrate meal transiently increases insulinemia and fullness but substrate metabolism is unaffected.

**Keywords:** lipid metabolism; calcium; satiety response; hunger; insulin; exercise; randomized-controlled trial; fat oxidation

## 41    **Introduction**

42    Achieving an optimal body composition is important for many athletes (both elite and  
43    recreational), and is achieved via manipulation of both diet and exercise programmes.  
44    Calcium intake is associated with reduced fat mass (Schrager 2005), and can accelerate  
45    fat loss under energy restriction (Zhu et al. 2013) therefore possibly providing an  
46    opportunity for nutritional intervention. This relationship between calcium intake and  
47    body fat may be accounted for, in part, by increased faecal fat excretion (Christensen et  
48    al. 2009), whole-body lipid oxidation (Gonzalez et al. 2012a) and/or appetite regulation  
49    (Major et al. 2009; Bellisle and Tremblay 2011; Gilbert et al. 2011; Ping-Delfos and  
50    Soares 2011).

51

52    Most literature to date has focussed on chronic calcium supplementation, and less is  
53    known about the acute metabolic impact of calcium ingestion. Elevated postprandial  
54    lipid oxidation (Soares et al. 2004; Cummings et al. 2006; Ping-Delfos and Soares  
55    2011) and non-esterified fatty acid (NEFA) availability (Soares and Chan She Ping-  
56    Delfos 2008) have been observed following ingestion of high calcium and vitamin D  
57    meals, in older, overweight/obese populations. This suggests calcium can overcome part  
58    of the postprandial suppression of fat oxidation when carbohydrate-containing meals are  
59    consumed (Gonzalez et al. 2013). However these investigations were performed at rest,  
60    and exercise metabolism in response to acute calcium intake has received little interest.

61

62    To date, only one study has investigated this specifically (White et al. 2006). Trained  
63    female runners were provided with 5 g/carbohydrate/kg body mass in the 4 h prior to

exercise from 2 meals, the second of which (consumed 1 h prior to exercise) was a test drink either high (500 mg) or low (80 mg) in calcium. No differences were observed in lipid oxidation during exercise. However, evidence from others indicates that NEFA concentrations diverge at 3 h post-consumption of test meals differing in calcium content (Cummings et al. 2006; Ping-Delfos and Soares 2011). As such, the 1 h between test-meal consumption and exercise may not have been long enough to allow for any calcium-related changes. Furthermore, the period of fast prior to test drink consumption was not standardised between-individuals. Therefore we aimed to provide a test meal 3 h prior to exercise and with a lower carbohydrate load, yet still in accordance with sports nutrition guidelines (Burke et al. 2011), to examine the effect of acute calcium intake on subsequent exercise metabolism with diet and activity standardised for 24 h prior to trials.

Considering appetite regulation, some have theorised about calcium specific appetite (Tordoff 2001) which may play a role in energy intake. Evidence is also available that 6 months of milk supplementation augments fasted fullness sensations (Gilbert et al. 2011) and ingestion of a high-calcium and vitamin D breakfast can reduce subsequent 24-h energy intake.

Accordingly, the objective of this study was to investigate the impact of acute calcium intake on postprandial subjective appetite ratings and substrate metabolism during rest, exercise and recovery.

## 87    **Materials and Methods**

### 88    **Participants**

89    Based on previous findings of a 2.7 g/h (5.4 g for 2 h) difference in lipid oxidation with  
90    a high calcium and vitamin D breakfast (Ping-Delfos and Soares 2011) and our own  
91    typical error of this postprandial measure equating to 3.37 g over a 2 h period (Gonzalez  
92    et al. 2012b) it was calculated that a sample size of 8 would provide statistical power  
93    above 80% with an alpha level of 0.05. In order to account for dropouts, the aim was to  
94    recruit a sample of 10.

95

96    Following completion of informed written consent, ten physically active males were  
97    recruited from the student and staff population at Northumbria University. One  
98    participant withdrew from the study due to time commitments and therefore nine  
99    participants are included in the analysis. Their age, height, body mass, BMI, peak  
100    oxygen uptake ( $V_{O2peak}$ ) and habitual calcium intakes were (mean  $\pm$  SD)  $25 \pm 4$  years,  
101     $180.8 \pm 7.8$  cm,  $79.6 \pm 7.1$  kg,  $24.4 \pm 2.5$  kg/m<sup>2</sup>,  $55.1 \pm 5.4$  ml/kg/min and  $1047 \pm 487$   
102    mg/d, respectively. Eligibility criteria included, young ( $< 35$  y), non-obese (BMI  $< 30$   
103    kg/m<sup>2</sup>), self-reported physically active ( $>30$  min of structured exercise, 5 times/week)  
104    and no known metabolic or gastrointestinal diseases or food allergies. The protocol was  
105    approved by the School of Life Sciences Ethics Committee at Northumbria University.

106

### 107    **Preliminary Measurements**

108    Participants undertook two preliminary tests to establish 1) the relationship between  
109    oxygen uptake and running speed on a flat treadmill using a 16-min test, and 2) their

VO<sub>2peak</sub> using an incremental treadmill test whereby the gradient was increased by 1%/min to exhaustion as previously described in full detail (Williams et al. 1990). On the same day, participants were familiarised with the visual analogue scales (VAS) to later assess subjective appetite sensations in main trials. A food frequency questionnaire, previously validated, and used in athletic populations (Taylor et al. 2009; Bescos Garcia and Rodriguez Guisado 2011), was completed to estimate habitual calcium intake.

## Experimental Design

Participants completed two trials in a randomized (randomization performed by J.T.G. using an electronic statistical package), double-blind, crossover design separated by  $\geq 7$  d which consisted of a control (CON) and high milk-calcium (CAL) trial. All trials were performed under similar laboratory conditions (mean  $\pm$  SEM; Temperature:  $20.5 \pm 1.3$  and  $20.2 \pm 1.1$  °C; Humidity:  $49 \pm 3$  and  $48 \pm 3$  %; Pressure:  $1021 \pm 7$  and  $1021 \pm 9$  mbar for CON and CAL trials, respectively; all  $P > 0.05$ ). Food and fluid diaries were kept for the day preceding the first trial and participants were instructed to replicate this for all subsequent trials. Participants were asked to avoid all foods containing dairy in the final meal prior to trials. Alcohol, caffeine and vigorous activity were prohibited for 24 h prior to trials. Compliance to diet and physical activity was assessed by self-completed food diaries and verbal questioning.

On the morning of the trials, participants arrived in the laboratory at 0730 after a 10-14 h fast and a cannula was inserted into an antecubital vein for blood sampling. After

baseline expired gas samples and VAS, participants were served breakfast which consisted of honey nut cornflakes (Tesco, Dundee, UK), skimmed milk (Cravendale, Arla Foods, Denmark) and 100 ml water. The breakfast provided 1.5 g carbohydrate/kg body mass in accordance with current sports nutrition guidelines for pre-exercise meals (Burke et al. 2011). The breakfasts were identical in energy ( $600 \pm 53$  kcal), protein ( $17 \pm 2$  g), carbohydrate ( $119 \pm 11$  g), fat ( $6 \pm 1$  g), and vitamin D ( $7 \pm 1$   $\mu$ g) content. On CAL trials, milk-extracted calcium (Capolac®, Arla Foods Ingredients a.m.b.a., Denmark) was added to the milk to increase calcium content from 3 to 9 mg/kg body mass (CON:  $238 \pm 21$  mg, CAL:  $716 \pm 74$  mg). The calcium powder was completely soluble in milk and the quantities used to increase the calcium content of the meal resulted in negligible increases in protein, carbohydrate and fat (all  $< 0.5$  g) and sodium, magnesium, chloride and potassium (all  $< 90$  mg). Water consumption was *ad libitum* during the postprandial period in the first trial and water intake was replicated for the subsequent trial. Following a 3 h postprandial breakfast period, participants ran on a treadmill at 60%  $\text{VO}_{2\text{peak}}$  for 60 min (Figure 1).

Immediately following exercise, participants ingested 500 ml of chocolate milk as a test drink (Yazoo, Campina Ltd, West Sussex, UK) providing 1500 kJ (18, 63, and 19 % of energy from protein, carbohydrate and fat, respectively) followed by a 90 min recovery period. Chocolate milk was chosen due to its effectiveness as a post-exercise recovery drink (Thomas et al. 2009) and also as a means to investigate potential second-meal effects following the high-calcium breakfast (Soares et al. 2004; Ping-Delfos and Soares 2011).



On a separate day, 12 participants (all of the original 10 participants in the study, plus an additional 2 physically active males) performed a triangle taste test to ensure that there was no detectable difference in taste between the breakfasts. In brief, participants tasted three breakfasts, one of which differed from the other two in calcium content. Participants were asked to identify the odd one out of the three. As only 3 out of 12 were correct, this was below the 33% considered to be correct by chance.

#### **Anthropometric Measurements**

Body mass was determined to the nearest 0.1 kg using balance scales (Seca, Birmingham, UK) upon arrival to the laboratory, where participants wore only light clothing. Height was measured to the nearest 0.1 cm using a stadiometer (Seca, Birmingham, UK).

#### **Blood Sampling and Analysis**

10 ml blood samples were collected at baseline, at 15, 30, 45, 60, 90, 120 and 180 min following breakfast consumption (Figure 1). All samples apart from the time point during exercise were obtained whilst participants were supine to control for changes in plasma volume with posture. Following exercise and test drink ingestion, additional 1 ml samples were collected at 5, 10, 15, 20, 25, 30, 45, 60 and 90 min where blood glucose was determined immediately by a glucose analyzer (Biosen C\_line, EKF Diagnostics, Magdeberg, Germany). Of the 10 ml samples, a 20 µl capillary tube was filled with whole blood and used to determine blood glucose concentrations, and the remaining whole blood was allowed to stand for 30 min in a non-anticoagulant tube

before being centrifuged at 3000 g and 4°C for 10 min. Aliquots of serum were stored at -80°C for later determination of NEFA (WAKO Diagnostics, Richmond, VA) and insulin (IBL International, Hamburg, Germany) concentrations in duplicate. Intra-assay coefficients of variation were 8.4% and 3.0% for NEFA and insulin, respectively. As exercise of a similar, and indeed greater, intensity and duration has been shown not to result in changes in plasma volume (Broom et al. 2007; Burns et al. 2007; Martins et al. 2007; Broom et al. 2009), it was decided that there was no need to adjust analyte concentrations following exercise and test drink ingestion.

### **Energy Expenditure and Substrate Oxidation**

Substrate metabolism was calculated assuming negligible protein oxidation, with rates of oxygen consumption ( $\text{VO}_2$ ) and carbon dioxide production ( $\text{VCO}_2$ ) using stoichiometric equations and was adjusted during exercise to account for the contribution of glycogen to metabolism (Jeukendrup and Wallis 2005):

$$\text{Rate of lipid oxidation at rest and during exercise (g/min)} = (1.695 \times \text{VO}_2) - (1.701 \times \text{VCO}_2) \quad (1)$$

$$\text{Rate of carbohydrate oxidation at rest (g/min)} = (4.585 \times \text{VCO}_2) - (3.226 \times \text{VO}_2) \quad (2)$$

$$\text{Rate of carbohydrate oxidation during exercise (g/min)} = (4.585 \times \text{VCO}_2) - (2.962 \times \text{VO}_2)$$

(VO<sub>2</sub> and VCO<sub>2</sub> expressed as L/min) (3)

203

204 Energy expenditure was calculated based on lipids, glucose and glycogen providing  
205 40.81, 15.64 and 17.36 kJ/g, respectively. At rest, calculations were based on glucose  
206 providing all of the carbohydrate for metabolism, whereas during moderate intensity  
207 exercise carbohydrate oxidation is met by both glucose and glycogen providing a 20 and  
208 80% contribution, respectively (Jeukendrup and Wallis 2005).

209

210 The assumption of negligible protein oxidation was unlikely to affect the estimations of  
211 substrate oxidation, as calcium (from dairy or as calcium citrate) does not influence  
212 postprandial protein oxidation (Cummings et al. 2006). For further clarification  
213 however, the respiratory exchange ratio (RER) is also reported.

214

215 Expired gas samples were collected using an online gas analysis system (Metalyzer 3B,  
216 Cortex, Germany) calibrated using gases of known concentrations and a 3 L syringe.  
217 Participants wore a facemask and after a 5 min stabilisation phase, 10 min samples were  
218 obtained and averaged at baseline, every 30 min after breakfast and test drink  
219 consumption in line with best practice methods (Compher et al. 2006). Expired gas was  
220 continuously sampled throughout exercise and averaged over each 5 min period  
221 ignoring the first 5 min to allow for steady-state values. Heart rate was also determined  
222 at 15 min intervals throughout exercise via short-range telemetry (Polar Electro UK  
223 Ltd., Warwick, UK).

224

## **Subjective Ratings**

Paper based, 100 mm VAS were completed at baseline, immediately following breakfast and every 30 min thereafter. Questions asked were used to determine hunger, fullness, satisfaction, prospective food consumption (Flint et al. 2000).

## **Statistical Analysis**

Due to difficulties with blood collection in one participant, data for blood analytes are presented from 8 participants. Subjective appetite ratings and blood analyte concentrations were converted into time-averaged area under the curve (AUC) using the trapezoidal rule.

Data were tested for normal distribution using the Anderson-Darling normality test and data not displaying normal distribution were log-transformed prior to statistical analysis. Paired t-tests were used to determine differences at baseline, and differences in total lipid oxidation, carbohydrate oxidation, energy expenditure, and AUCs between trials. Two-way repeated measures ANOVA (trial x time) were used to detect differences for all variables. Holm-Bonferroni step-wise post-hoc test was utilised to determine the location of the variance. Pearson product-moment correlation coefficients were used to determine relationships between insulin concentrations and appetite sensations and between habitual calcium intake and postprandial responses. Statistical significance was set at  $P < 0.05$ . All results are presented as mean  $\pm$  SEM

## Results

### Energy Expenditure and Substrate Oxidation

There were no differences in baseline energy expenditure, lipid or carbohydrate oxidation rates, or RER (all  $P < 0.05$ ). No significant interaction effects (trial x time) were detected for either energy expenditure, lipid oxidation or carbohydrate oxidation (all  $P > 0.05$ ). After breakfast consumption, energy expenditure rose to a similar peak in both CON and CAL trials ( $2.0 \pm 0.3$  and  $2.1 \pm 0.3$  kcal/min, respectively;  $P > 0.05$ ) and lipid oxidation was suppressed to a similar extent in both trials (postprandial change from baseline:  $-0.05 \pm 0.04$  and  $-0.04 \pm 0.03$  g/min for CON and CAL, respectively;  $P > 0.05$ ). Lipid oxidation rose throughout exercise with no differences between trials (Figure 2).

There were no differences in total energy expenditure, lipid or carbohydrate oxidation in the postprandial period following breakfast, during exercise, or during recovery (Table 1). There was also no difference in mean heart rate during exercise (CON:  $137 \pm 6$ , CAL:  $137 \pm 6$  beats per min;  $P > 0.05$ ).

No significant differences between trials were detected in RER following breakfast, during exercise, or recovery (Table 1; all  $P > 0.05$ ).

### Blood Glucose, Serum NEFA and Insulin Concentrations

There were no significant differences between trials in baseline glucose, NEFA or insulin concentrations (all  $P > 0.05$ ).

No significant interaction (trial x time) effects were observed in blood glucose or serum NEFA concentrations at any time point ( $P > 0.05$ ; Figure 3). The 180 min AUC for NEFA was  $0.08 \pm 0.02$  and  $0.09 \pm 0.03$  mmol/L for CON and CAL trials, respectively ( $P > 0.05$ ). Peak insulin concentrations were  $339 \pm 45$  and  $351 \pm 36$  pmol/L in the CON and CAL trials, respectively ( $P > 0.05$ ; Figure 3). The insulin AUC for the first hour was  $10 \pm 3\%$  greater in CAL vs. CON ( $P = 0.02$ ; Figure 4), but when this was extended to the full 180 min AUC, there was no difference between trials ( $148 \pm 46$  and  $157 \pm 14$  pmol/L for CON and CAL trials, respectively;  $P > 0.05$ ).

No differences in blood variables were detected during the recovery period (Table 2,  $P > 0.05$ )

### **Subjective Ratings**

No significant interaction (trial x time) effects were observed for any subjective appetite ratings (all  $P > 0.05$ ). There was however, a trend for an interaction effect (trial x time), and a significant main effect (trial) detected for fullness sensations ( $P = 0.085$  and  $P = 0.007$ , respectively).

The fullness AUC for 0-60, 0-120 and the full 0-180 min postprandial breakfast periods were 16, 14 and 11% greater in CAL vs. CON, respectively (Figure 5 and Table 2;  $P = 0.008$ ,  $0.01$  and  $0.04$ , respectively). The percentage change in insulin AUC for the 0-60 min, showed strong positive relationships with the percentage change in fullness AUC for the same time period ( $r = 0.95$ ,  $P < 0.01$ ) and for the full 180 postprandial period ( $r = 0.92$ ,  $P < 0.01$ ). Neither hunger, nor satisfaction, nor prospective consumption differed significantly between trials (Figure 5; all  $P > 0.05$ ). No differences in any appetite sensations were observed during recovery ( $P > 0.05$ ).

#### **Relationships with habitual calcium intake**

Habitual calcium intake as assessed by the food frequency questionnaire was not associated with any of the high-calcium meal induced-changes in postprandial variables (All  $P > 0.05$ ).

#### **Discussion**

The main findings of this study demonstrate that increasing the dairy calcium content of a meal does not affect substrate oxidation or energy expenditure before, during or after exercise in physically active males, but transiently increases feelings of fullness and insulin concentrations.

Acute high-calcium and vitamin D intake has been shown to increase postprandial lipid oxidation in overweight/obese subjects on two occasions (Cummings et al. 2006; Ping-

Delfos and Soares 2011). The present study found no significant difference in either postprandial, or exercise lipid oxidation in recreationally active males. Magnitude based inferences suggest a 95% and 92% chance of merely a trivial difference in postprandial and exercise lipid oxidation, respectively. This corroborates the findings in female runners (White et al. 2006). Both these exercise studies involved consumption of high carbohydrate loads prior to exercise (1.5 and 5 g/kg body mass), in accordance with sports nutrition guidelines (Burke et al. 2011). Carbohydrate intake blunts NEFA availability and lipid oxidation at both rest and during exercise (Gonzalez et al. 2013) via insulin-induced suppression of lipolysis. It may be that the action of calcium was eclipsed by the large carbohydrate loads' blunting of NEFA availability. Indeed, NEFA were still suppressed to ~0.05 mmol/L when exercise began (Figure 3C).

Notwithstanding this, others have reported greater rates of lipid oxidation in the postprandial period following moderate (~70 g) carbohydrate loads (Cummings et al. 2006; Ping-Delfos and Soares 2011) with similar levels of calcium intake (difference between CAL vs. CON: ~400 mg in Ping-Delfos & Soares (2011) and ~500 mg in the current investigation) suggesting that calcium may partially overcome the suppression of lipid oxidation after food intake. The 60 min postprandial rise in insulin concentrations seen in the present study (~250%) was less than the ~300% reported by Ping-Delfos & Soares (2011), and the 180 min postprandial change in insulin concentrations were similar to the full 6 h postprandial change in insulin concentrations (both ~230 pmol/L) displayed by Cummings et al. (2006). Thus, with similar postprandial insulinemia and calcium doses, some studies show increased NEFA availability and lipid oxidation with high calcium meals, whereas the present study did not. The likely superior insulin sensitivity of the recreationally active, lean individuals



[although 33 % of participants had a BMI of  $> 25 \text{ kg/m}^2$ , their adiposity was likely low given the high  $\text{VO}_2\text{peak}$  (Janssen et al. 2004)] in the current study may have resulted in a more pronounced insulin-induced suppression of lipolysis (Frayn et al. 1997) compared to the overweight populations used previously (Cummings et al. 2006; Ping-Delfos and Soares 2011). We cannot exclude the possibility that differences in expired gas collection (facemask vs. ventilated hood) underlie the discrepancy between the present study, and that of others (Cummings et al. 2006; Ping-Delfos and Soares 2011). Although good practise guidelines suggest there is negligible difference between the use of facemasks and ventilated hoods (Compher et al. 2006).

In previous studies, postprandial responses to high-calcium meals included lessened suppression of lipid oxidation in the presence of similar insulinemia (Cummings et al. 2006; Ping-Delfos and Soares 2011), and the present study demonstrates similar lipid oxidation with higher (albeit modest and transient) insulinemia. Given that this represents a degree of reduced insulin action and metabolic inflexibility, the underlying potential mechanisms deserve discussion. Largely *in vitro* data have suggested that parathyroid hormone and calcitriol are the major regulators of lipolysis and lipid metabolism in response to calcium intake (Zemel 2004; Gonzalez and Stevenson 2012a; Soares et al. 2012). *In vivo* evidence pertaining to these mechanisms in humans however, does not support this. Elevated NEFA availability and lipid oxidation is evident without diverging parathyroid hormone concentrations (Melanson et al. 2005; Ping-Delfos and Soares 2011), and marked changes in calcitriol concentrations do not influence whole body lipid oxidation, circulating NEFA availability or the expression of lipolytic genes in adipose tissue (Boon et al. 2006). Future work is needed to substantiate not only the efficacy of calcium in modulating lipid oxidation but also the

underlying mechanisms. The gastrointestinal peptides glucose-dependent insulintropic peptide (GIP) and glucagon-like peptide-1 (GLP-1) may offer some insight. The concentration of these peptides can be enhanced by calcium co-ingestion (Gonzalez and Stevenson 2013), they have been implicated in lipolysis (Timper et al. 2013) and, when manipulated over a period of days, increase lipid oxidation (Boschmann et al. 2009). This makes it tempting to speculate that repeated calcium ingestion with the associated increased exposure to GIP and GLP-1 may lead to a shift in substrate metabolism over a period of days (as is the case with pharmaceutical manipulation (Boschmann et al. 2009)) which is easier to detect than after acute ingestion.

The present study demonstrates that increasing the calcium content of a meal can increase insulin concentrations and feelings of fullness following consumption, whilst other appetite sensations were not significantly affected. We have previously reported that fullness is more susceptible to interventions than hunger and other appetite sensations (Gonzalez and Stevenson 2012b). It may be that fullness is more intimately linked with physiological sensations (such as gastric distension and the metabolic and hormonal milieu), whereas other appetite sensations are, additionally, influenced by psychological factors (Stubbs et al. 2000). Supporting this, the difference in insulinemia between trials was strongly related to the change in fullness sensations. Similar effects have been reported previously (Flint et al. 2007) and suggest that insulin plays a strong role in postprandial fullness sensations. It should however, be acknowledged that the changes observed were relatively small and of a short duration and thus their impact in the real-life situation needs clarification.

The acute appetite effects of calcium have previously been inconsistent, with reduced energy intake seen by some using a realistic meal design (Ping-Delfos and Soares 2011), but not by others with a very large (~4500 kJ; ~1075 kcal) energy load (Lorenzen et al. 2007). Also, to date, evidence of mechanisms in humans is sparse. Ping-Delfos et al. (2011) found no effect of increased calcium intake on appetite sensations, insulin or leptin concentrations, yet blood was only sampled every hour and, as we have observed a transient effect on insulin and fullness, these authors may have missed the divergence in insulin concentrations. Lorenzen et al. (2007) took more frequent blood samples following meal ingestion. Nevertheless, no effect was observed on AUCs of insulin, or any other appetite-related hormones (namely cholecystokinin, ghrelin, glucagon-like peptide-1 and peptide YY). A potential caveat of Lorenzen et al. (2007) was the energy content of the test meal, which provided 50% of daily energy requirements. This energy load may have caused such large perturbations to postprandial hormone concentrations, that subtle effects were masked.

Given that there was no significant relationship between the habitual calcium intake and the responses seen, it would suggest that the acute effects of calcium are independent of habitual intake, although to corroborate this a larger study with a wide range of intakes would be necessary.

It may be regarded as a limitation with the present study that subsequent energy intake (ie. at lunch) was not measured, and therefore interpretations as to the impact of fullness on energy balance are difficult. However, this study provides novel data by demonstrating that milk-calcium can transiently increase satiety concomitant with

higher insulin concentrations thereby revealing one of the prospective mechanisms by which calcium intake may affect appetite and provides a platform from which future studies can investigate the effectiveness of calcium intake on metabolism and appetite in athletic and physically active populations. Furthermore, to the best of the authors' knowledge this is the first double-blind study to investigate the acute effects of calcium ingested with a high-calcium meal on substrate metabolism and appetite. It is worthy to note that the calcium supplement was undetectable, and therefore the influence of taste and palatability can be discounted (Bellisle et al. 2012) and differences can be attributed to the calcium content *per se*.

In conclusion, the present study indicates that, increasing the dairy calcium content of a meal from 0.3 to 0.9 g/kg body mass does not affect substrate metabolism at rest during exercise, or recovery in moderately trained males. However, increasing the calcium content of a meal can augment postprandial fullness, possibly (in part) via greater circulating insulin concentrations. It should be acknowledged that this augmentation is modest and transient. More research is required to understand the long-term implications of these findings, principally regarding energy balance and metabolism.

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**Table 1.** Energy expenditure and substrate metabolism in the breakfast postprandial period, exercise period and recovery postprandial period.

Trial	Breakfast period				Exercise period				Recovery period			
	(0-180 min)				(180-240 min)				(240-330 min)			
	EE	CO	LO		EE	CO	LO		EE	CO	LO	
	(kcal)	(g)	(g)	RER	(kcal)	(g)	(g)	RER	(kcal)	(g)	(g)	RER
CON												
Mean	1436	47.9	16.8	0.85	2951	100.4	30.5	0.87	778	15.4	13.2	0.79
SEM	24	1.5	0.6	0.01	47	2.7	0.8	0.01	12	1.0	0.4	0.02
CAL												
Mean	1446	46.6	17.5	0.84	2964	99.7	31.1	0.86	802	15.6	13.7	0.79
SEM	21	1.9	0.6	0.02	51	3.0	1.0	0.01	14	0.8	0.5	0.02

EE, energy expenditure; CO, carbohydrate oxidation; LO, lipid oxidation; CON, control; CAL, high-calcium. No significant differences were detected between trials by paired t-tests.

**Table 2.** Blood glucose concentration and subjective appetite sensations in the breakfast postprandial period and recovery postprandial period.

Time Period	Trial	Time-Averaged AUC				
		Blood glucose (mmol·L <sup>-1</sup> )	Hunger	Fullness	Satisfaction	Prospective consumption
Breakfast Period (0-180 min)	CON					
	Mean	4.44	36	59	58	41
	SEM	0.32	6	7	6	7
	CAL					
	Mean	4.55	33	63*	59	42
	SEM	0.23	5	6	5	6
Recovery Period (240-330 min)	CON					
	Mean	4.60	49	41	41	54
	SEM	0.12	5	5	4	5
	CAL					
	Mean	4.64	51	38	38	57
	SEM	0.14	4	5	4	5

AUC, Area under the curve; CON, control; CAL, high-calcium. \*Significantly different to CON,  $P < 0.05$  as detected by paired t-tests.

**Figure Captions:**

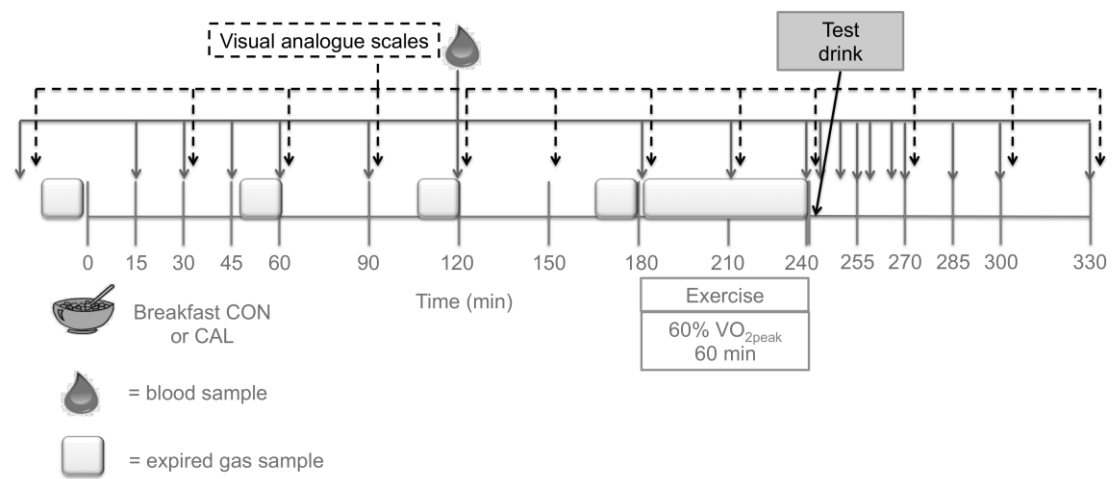
**Figure 1** – Schematic representation of main trial protocols. Solid arrows indicate blood samples; dashed arrows represent visual analogue scales.

**Figure 2** – Rates of lipid oxidation (**A**), carbohydrate oxidation (**B**) and energy expenditure (**C**) during the control (●) and high-calcium (○) trials. Values are means ± SEM.

**Figure 3** – Blood glucose (**A**), serum insulin (**B**) and serum non-esterified fatty acid (NEFA; **C**) concentrations for the first 240 min of control (●) and high-calcium (○) trials. Values are means ± SEM.

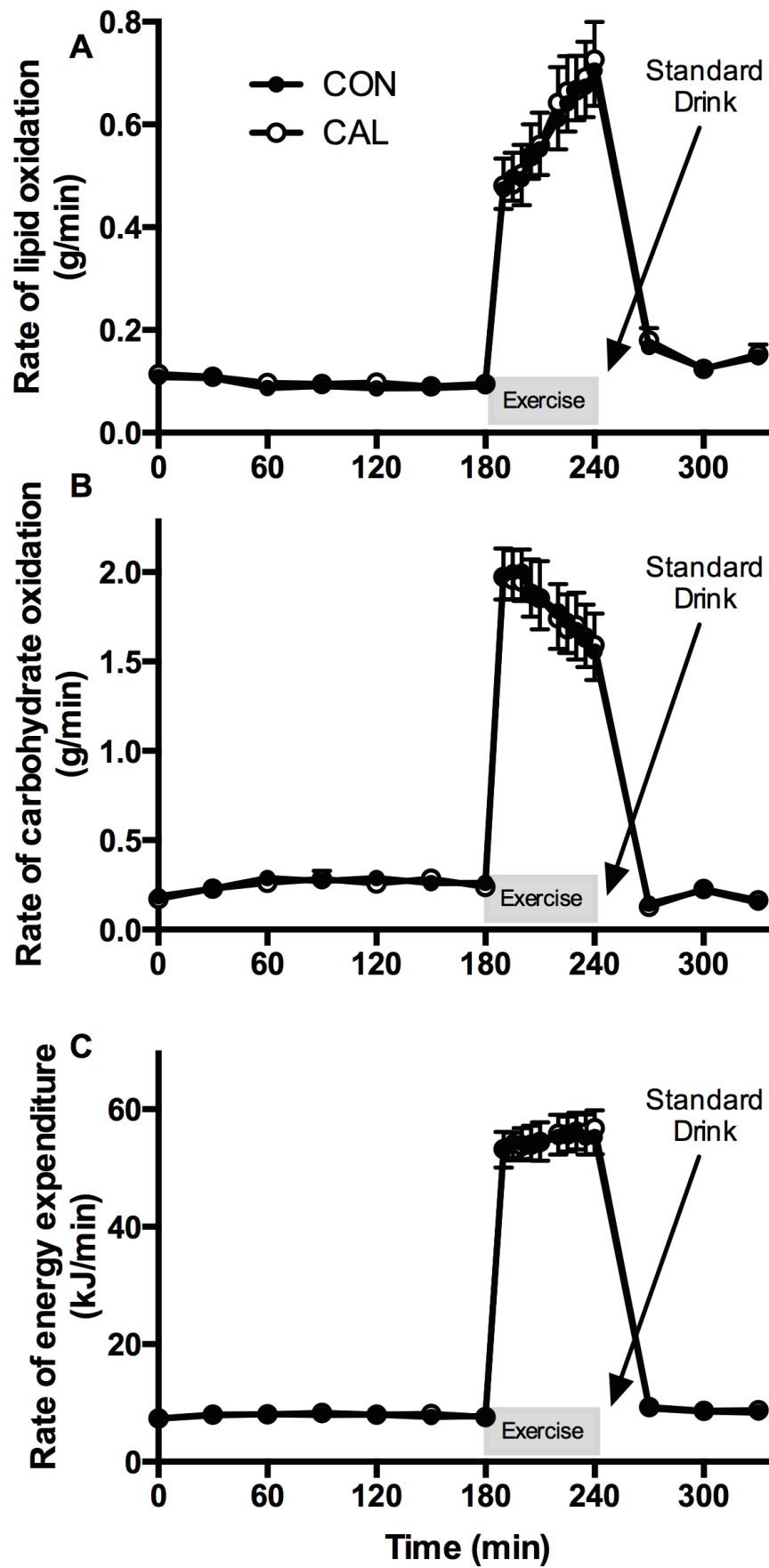
**Figure 4** – Serum insulin time-averaged area under the curve (AUC) for the first 60 min of control (CON) and high-calcium (CAL) trials. Both individual data (○) and group means (●) shown. \*Significantly different to CON,  $P < 0.05$ .

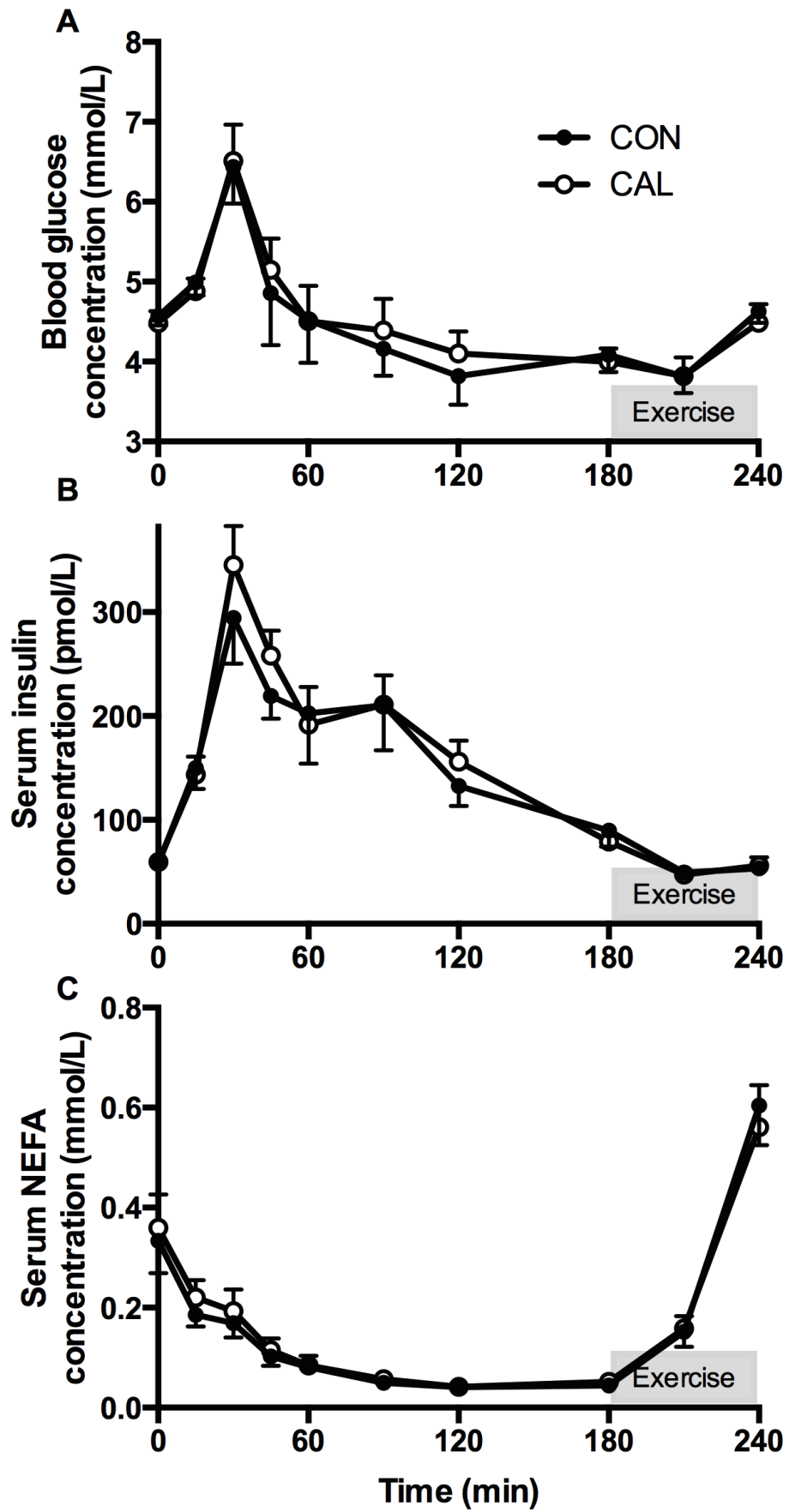
**Figure 5** – Hunger (**A**), fullness (**B**), satisfaction (**C**) and prospective consumption (**D**) for the first 240 min of control (●) and high-calcium (○) trials. Values are means ± SEM.



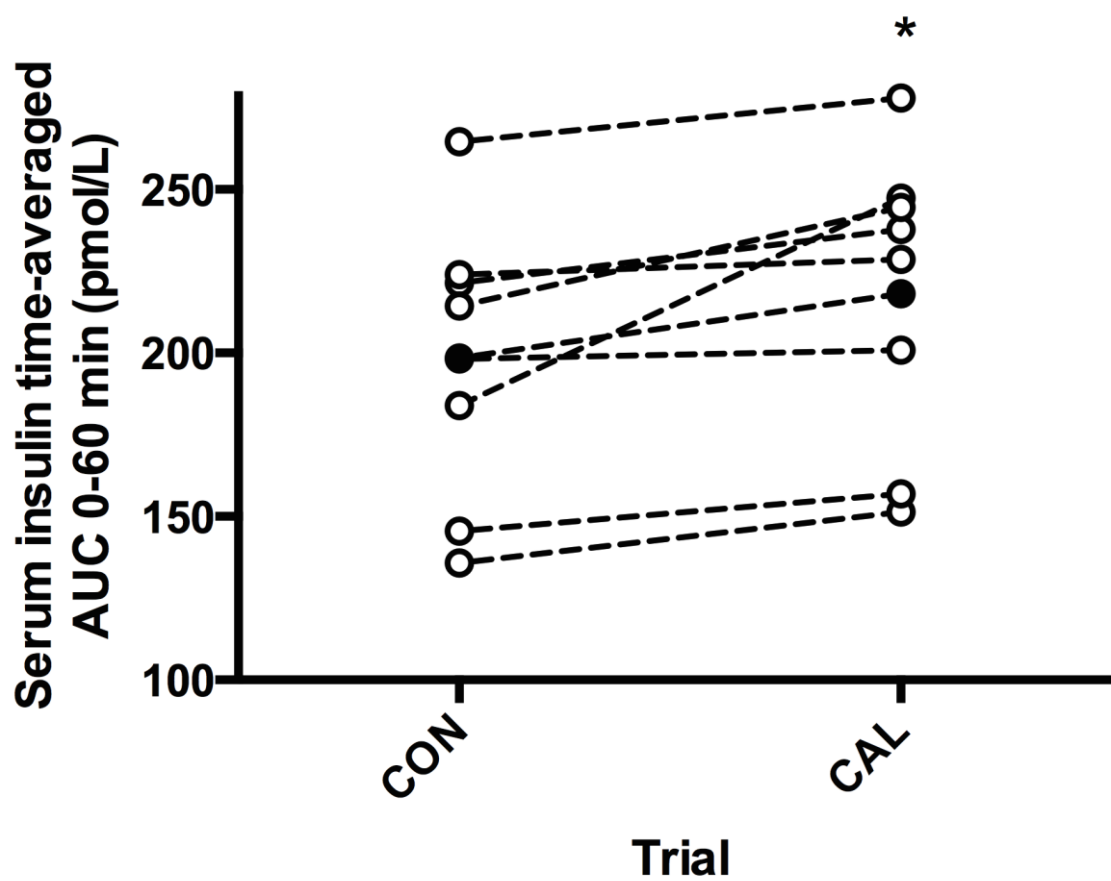
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